REMARKS

I. Preliminary Remarks

Applicants wish to thank the Examiners for the courtesy of the interview conducted on December 6, 2006 at which Examiner Schultz was personally present and Examiner Wessendorf was present telephonically. The background of prior art two-hybrid systems for testing protein-protein interactions was discussed as were the variety of possible two-hybrid systems. In addition, the mechanism of Applicants' assay methods was also discussed. Finally, several possible amendments to the claims which had been proposed by Examiner Wessendorf prior to the Interview were discussed. Applicants, through their attorney, agreed to make several of the proposed amendments while the Examiners agreed that the remaining proposed amendments were not required in order to place the claims in condition for allowance as will be discussed below.

The Examiners further agreed at the Interview that the concept of the invention is broader than the identity of any particular two-hybrid construct. In addition, the Examiners agreed that the use of claim language excluding antibody/antigen binding was neither new matter nor was it an improper "negative limitation." The Examiners further agreed that the claimed invention was novel and unobvious over the cited references but stated that additional prior art searching might be needed to confirm that there did not exist different art which would render the claims unpatentable.

The following amendments recite that the conditions are "reducing conditions" and specify that a "reporter gene [that] is expressed...in the host cell." The amendments further recite that the interaction of the transcriptional activation domain with the DNA binding domain "results in the growth or identification of cells indicating that the intrabody frameworks or intrabodies are stable and solutle under reducing conditions...." These amendments are supported by the disclosure and comport with the suggestions of the Examiners. As such they are believed to obviate the outstanding rejections and place the claims in order for allowance.

II. The Subject Matter of the Invention

The present invention is directed to a method of identifying intrabody frameworks and intrabodies which are soluble and stable under selected conditions in the intracellular environment. The invention is based on the finding that the solubility/stability of a fusion protein comprising a marker protein and an intrabody is dependent on the solubility/stability of the intrabody moiety. Thus, if the intrabody moiety is soluble and stable in the intracellular environment then the marker protein can be detected and cells expressing such a stable fusion protein can be selected.

The methods of the invention utilize the two hybrid system but do not involve any interaction between a scFv and its corresponding antigen (an antibody/antigen reaction) as do Visintin and Hoeffler.

III. Two Hyrbid Systems and the Prior Art

The two hybrid system is a method of testing for protein-protein interactions by testing for physical interactions, such as binding, between two proteins. One protein is termed bait and the other is prey. As noted in the Wikipedia entry, along with the Plant Physiology Online reference attached hereto as **Exhibit A**, the premise behind the two-hybrid test is the activation of a downstream reporter gene by binding of a transcription factor onto an upstream activating sequence (UAS). For purposes of two-hybrid screening, the transcription factor is split into two separate fragments, called Binding Domain (BD) and Activating Domain (AD). The BD is the domain responsible for binding to the UAS and the AD is the domain responsible for activation of transcription.

As noted in **Exhibit A**, the key to the two-hybrid screen is that in most eukaryotic transcription factors, the activating and binding domains are modular and can function in close proximity to each other without direct binding! This means that even though the transcription factor is split into two fragments, it can still activate transcription when the two fragments are indirectly connected and interact.

Thus, bait plasmids can be engineered so as to produce a protein product in which the BD fragment is fused to a bait protein. Prey plasmids are then engineered to produce a

protein product in which the AD fragment is fused onto the prey protein. The bait protein can be a known protein for which the investigator is attempting to identify new binding partners while the prey protein can be either a known protein or a random library protein. When the bait protein and the prey protein interact, the AD and BD of the transcription factor are indirectly connected and transcription of a reporter gene occurs. If the bait and prey proteins do not interact there is no transcription of the reporter gene. Typical reporter genes can encode for enzymes that allow synthesis of specific nutrients required by a host cell for growth or can encode a color marker.

The two-hybrid system was well known for use in a number of diagnostic systems including method for intrabody screening. For example, Visintin and Hoeffler rely upon the use of the two hybrid system for the isolation of intrabodies using an antibody/antigen interaction wherein the identification of the intrabodies is based on the interaction between the antibody and its corresponding antigen. In the case of the Visintin two-hybrid system illustrated in Fig. 1 of that reference, the antigen is linked to the LexA BD to form a bait protein while antibodies for screening against the antigen are linked to the VP16 AD to form a prey protein. When the antibody on the prey protein interacts with the antigen on the bait protein the AD and BD are brought into proximity with each other such that they activate transcription of a reporter gene. In the case of Visintin either a *LacZ* gene is activated resulting in a color signal or the *His*3 gene is activated allowing selection of cells in histidine negative media.

Applicants' method utilizes the two-hybrid system but does not determine whether an intrabody (antibody) is soluble and stable in the manner of the prior art. While the Visintin and Hoeffler assays will give a true positive signal if an intrabody is soluble, stable and specific for the target antigen, they will not give a signal (they report a false negative) when the intrabody is soluble and stable but is not specific for (lacks binding affinity for) the target antigen. As a consequence, many stable intrabodies which could be identified by Applicants' system are missed by the prior art systems.

As a means for better understanding the difference between the invention and the prior art the Examiners' attention is directed to the attached **Exhibit B** which depicts a comparison between the methods of Visintin and Hoeffler and that of Applicants.

IV. How Applicants' Invention Works

Applicants' method utilizes the two-hybrid system of protein interaction but does so in a manner different from that of Visintin and Hoeffler. In Fig. 1A of Applicants' application submitted herewith as **Exhibit C** a system is depicted in which the Gal4 DNA binding domain (DBD) serves at the bait while the prey is a fusion protein comprising the intrabody to be tested, the Gal4 transcriptional activation domain Gal4AD and a constant region (Gal11P). (See page 16, lines 33-35; para. [0075] in the specification. Stability of the prey fusion protein depends upon the stability and solubility of the scFv (intrabody) portion. If the intrabody is stable and soluble, the constant Gal11P domain interacts with the dimerization domain of Gal4 (residues 58-97) and the AD is brought into proximity to the BD thus activating transcription and expression of a reporter gene which in Fig. 1A can be either *His*3 allowing selection on a histidine negative medium or *Lac*Z resulting in a color signal.

Applicants' Fig. 1B submitted herewith as **Exhibit D** depicts a similar system wherein the bait protein comprised a LexA-Gal4 (58-97) protein and the prey protein comprised various intrabodies fused to the Gal4AD activation domain and the Gal11P constant region. (See page 17 lines 7-29; para. [0078-0080]) The intracellular stability and solubility of the prey fusion proteins depended on the scFv portion. Accordingly, only those stable and soluble scFv fusion proteins interacting with the LexA-Gal4 binding domain were able to activate reporter gene expression (either *His*3 or *Lac*Z).

Applicants' method determines whether the intrabody is stable and soluble but is not limited to whether the intrabody is specific for a particular antigen.

V. The Outstanding Rejections

Claims 31, 33-38 and 42-47 stand rejected over 35 U.S.C. §112 (first paragraph) for lack of written description on the basis that use of the term "transcriptional activation **domain**" introduces new matter into the case. Those claims are also rejected for use of the phrase "**not dependent upon the presence of the antigen for which the intrabody is specific**" as also introducing new matter.

Claims 31, 33-38 and 42-47 stand rejected over 35 U.S.C. §112 (second paragraph) as being indefinite. In paragraph (1) the Examiner asks whether the cell contains or is transformed with a DNA binding domain. The Examiner also questions how the interaction of the transcriptional activation domain with the DNA binding domain expresses a marker protein.

Claim 31 stands rejected for use of the term "selected conditions" on the basis that "the specification teaches only under reducing conditions that the intrabodies are not stable and soluble."

Claim 31 stands rejected on the basis that the negative limitation "...not dependent upon the presence of the antigen for which the intrabody is specific" is unclear.

Claims 31, 33, 35-38 and 43-47 stand rejected under 35 U.S.C. §103(a) over Visintin or Hoeffler.

Claim 42 stands rejected under 35 U.S.C. §103(a) over Visintin in view of Ptashne et al., (20040014036).

Claim 34 stands rejected under 35 U.S.C. §103(a) over Visintin in further view of Martineau or Nolan US 6,153,380.

Claims 31, 33-38, and 43-47 (of USSN 09/750,424) are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over copending USSN 10/169,179. Conversely, claims 19-23 and 39 of USSN 10/169,179 are provisionally rejected for obviousness-type double patenting over the present USSN 09/750,424 in an outstanding Office Action.

VI. The Examiner's Proposed Claims

In a telephone conference with Examiner Wessendorf on August 22, 2006 the Examiner proposed entry of a set of claims which were said to address the rejections which were then made in the currently outstanding Office Actions.

The Examiner suggested that such amendments would resolve the outstanding issues with respect to 35 U.S.C. §112 (first and second paragraphs) as well as the issues under 35 U.S.C. §103 in USSN 09/750,424 and that corresponding amendments would obviate the

rejections in related application USSN 10/169,179. Specifically, the Examiner suggested that:

1) Substitution of "reducing" for "selected" conditions would comport better with the disclosure.

2) Recitation of a "transcriptional activation dimerization domain" was supported by the disclosure while the currently recited "transcriptional activation domain" is not.

3) Substitution of "reporter gene that is expressed" for "marker protein the expression of which is mediated" would comport better with the disclosure.

4) Substitution of "binding" for "interaction" between the transcriptional activation domain and the DNA binding domain would be more technically accurate.

5) Recitation that the DNA binding domain is present "in the host cell."

6) Recitation of "results in the growth of cells indicating that the intrabody frameworks or intrabodies are stable and soluble under reducing [rather than "selected"] conditions" would comport better with the disclosure.

7) The Examiner further suggested that recitation of "not dependent upon the presence of the antigen for which the intrabody is specific" could be deleted without adversely affecting the novelty and unobviousness of the claims.

8) The Examiner subsequently proposed that the claims be amended to recite "constant domain" that interacts with a "dimerization domain."

VII. How Applicants Propose Modifying the Proposed Claims

As discussed at the Interview, Applicants understand the rationale behind each of the Examiner's proposals and accept several of them (Nos. 1, 3 and 5) in order to expedite allowance of the present claims even if they do not agree that such proposals are necessary for patentability of the claims. Applicants do not accept several (Nos. 2, 4, 7 and 8) and propose modifying the other (No. 6) resulting in the claim amendments presented above.

Applicants accept each of Proposals 3 and 5 and further accept Proposal No. 1 to substitute "reduced conditions" for "selected conditions" although they continue to argue that method of the present invention is broadly applicable and is not limited to one set of adverse conditions such as reducing conditions. It is true that "reduced conditions" are those under which practice of the invention may be most useful but the ability of the inventive method to determine the stability and solubility of an intrabody is not limited to reducing conditions.

Applicants do not accept Proposal No. 2 to recite "transcriptional activation dimerization domain." This amendment was proposed by the Examiner on the basis that the specification did not support the recitation of "transcriptional activation domain." (See the Action dated October 23, 2006 at page 3.) Not only is "transcriptional activation domain" supported in the specification such as at page 12, line 21 [para. 0048]; page 14, lines 5-6 [para. 0055] and elsewhere but "transcriptional activation domain" is accepted as the proper terminology in the art. (See Visintin Fig. 1).

Applicants also do not accept Proposal No. 4 to substitute "binding" for "interaction" as describing the relationship between the transcriptional activation domain and the DNA binding domain. This is because, as noted above with reference to the two-hybrid system in Exhibit A, "the activating and binding domains are modular and can function in close proximity to each other without direct binding." Thus "binding" is incorrect descriptively and technically and "interaction" is the appropriate technical term.

Applicants submit that Proposal No. 6 is correct but incomplete as excluding disclosed and enabled marker systems. While certain types of marker systems, such as the *His*3 system, allow selection of cells in selective media such as histidine negative media; the use of selective media is only one way of practicing a two-hybrid system. As described in Exhibit A, Applicants' disclosure, dependent claim 34, and even in the Visintin reference, enzymatic markers such as a *LacZ* gene can be used to activate a color signal. See the "reporter genes" identified in each of Applicants' Figs. 1A and 1B (Exhibits C and D) as well as in Visintin Fig. 1. Thus, Applicants propose language reciting that the "reporter gene...results in the growth or identification of cells..."

With respect to Proposal No. 7, Applicants appreciate the Examiner's view that the recitation that the interaction between the transcriptional activation domain and with the DNA binding domain "is not dependent upon the presence of the antigen for which the intrabody is specific" is not necessary for patentability over the disclosure of Visintin and Hoeffler. Nevertheless, Applicants prefer retaining the current language which derives from language originally proposed by Supervisor Examiner Yang in distinguishing the two-hybrid system of the invention from those of the prior art. This language is supported by the disclosure at page 16, line 24 through page 17, line 5 [paras. 0073-0082] and Figs. 1a and 1b disclosing the "Quality Control System" of the invention.

Finally, with respect to Proposal No. 8 Applicants urge that the limitation to recitation of a "constant domain" which interacts with a "dimerization domain" is overly narrow because there exist numerous activation domain-dna binding domain (AD-DBD) systems at the time of the invention that would have been available to one of ordinary skill in the art.

As stated above, the Examiners agreed at the Interview that Applicants' acceptance proposals 1, 3 and 5 and 6 (as modified) resolved any remaining issues under 35 U.S.C. §112 (first and second paragraphs) for the reasons set out above.

VIII. Patentability Arguments

A. The Rejections of Claims 31, 33-38 and 42-44 Under 35 U.S.C. §103 Should Be Withdrawn.

As discussed previously, the prior art rejections over each of Visintin, or Hoeffler either alone or in light of the secondary references (Ptashne, Martineau and Nolan) should be withdrawn because both references rely upon the use of the two hybrid system wherein the claimed identification of the intrabodies is based on the interaction between the antibody and its corresponding antigen and does not determine whether an intrabody is soluble and stable as recited in the manner of Applicants' invention.

While the Visintin and Hoeffler assays will give a positive signal if an intrabody is soluble, stable and specific for the target antigen, they will not give a signal (they will report a false negative) when the intrabody is soluble and stable but is not specific for the target antigen. The Examiners' attention is directed to the attached **Exhibit B** which depicts a comparison between the methods of Visintin and that of Applicants.

The present Action argues that the claims do not distinguish over the imperfect prior art systems of Visintin and Hoeffler because the claims are comprising claims and therefore do not exclude the false negative results produced by the Visintin and Hoeffler systems!

The Office Action further states that:

> Simply because Visintin is silent in the assay of scfv's stability and solubility in the absence of antigen [the antigen for which the antibody is specific?] does not mean that one would not be able to determine such assay.

However, Visintin will tell one nothing about the solubility or stability of an intrabody in the "absence of antigen" for which the intrabody is specific. It would be

impossible to know from practicing Visintin in this manner whether an intrabody was stable or not!

The Office Action also states that:

> It is not apparent how the two-hybrid system employed by Visintin, as employed by applicants, would produce different results.

The two-hybrid system of Visintin would produce different results from Applicants' method because the Visintin method would fail to identify perfectly stable and soluble intrabodies only because those intrabodies are not specific for a selected antigen! The worker practicing the Visintin method would therefore miss identifying potentially valuable intrabodies! This is not to say that the Visintin system is without value; simply that Applicants' system is different from that disclosed by Visintin and will identify stable and soluble intrabodies that the Visintin method will not. As such, the claimed system represents a superior method of identifying stable and soluble intrabodies and an improvement in the art.

The Action also argues at page 8 that "At page 11727, col. 2 Visintin states that the paucity of functional intracellular scFv requires selection procedures that are based on intracellular action rather than in vitro antigen binding alone." This is true, but the sentence is then followed by the statement:

> We therefore have developed a general antibody-antigen two-hybrid assay system, in which a positive outcome (*e.g.*, activation of His3 or *LacZ*) depends on the interaction of scFv with target antigen under intracellular conditions. (Visintin page 11727, col. 2, lines 31-34, emphasis supplied)

> We therefore have developed an *in vivo* selection scheme for the isolation of intracellular scFv, based on their ability to bind antigen under conditions of intracellular expression. (Visintin, page 11727, col. 2, lines 56-58, emphasis supplied)

Visintin then discloses its "Model Selection" system described in the text bridging pages 11726 and 11727 relied upon the use of the AMCVp41/BTN116 bait with the scFvF8-VP16 fusion protein wherein the scFvF8 portion is an anti-AMCV antibody! This system not only is different from that practiced by Applicants but also teaches away from their system because of its reliance upon antibody/antigen binding.

For these reasons, the rejections under 35 U.S.C. §103 should be withdrawn.

B. The Rejections of Claims 31, 33-38 and 42-44 Under 35 U.S.C. §112 (first and second paragraphs) Should Be Withdrawn.

The rejections under 35 U.S.C. §112 (first and second paragraphs) should be withdrawn in light of the amendments of those claims and reasons set out above.

C. The Provisional Obviousness-type Double Patenting Rejection Should be Withdrawn in Light of the Terminal Disclaimer Submitted Herewith.

The provisional obviousness-type double patenting rejection should be withdrawn in light of the terminal disclaimer submitted herewith.

CONCLUSION

For the foregoing reasons it is submitted that each of claims 31, 33-38 and 42-44 should now be allowed. Should the Examiners wish to discuss any issues of form or substance in order to expedite allowance of the pending application, they are invited to contact the undersigned attorney of record, Jeffrey S. Sharp, at the number indicated below.

The Commissioner is authorized to charge any fee deficiency required by the paper to Deposit Account No. 13-2855.

Respectfully submitted,

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EXHIBIT A

EXHIBIT B

EXHIBIT C

EXHIBIT D